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OBSERVATIONS ON THE CHEMICAL COMPOSITION OF THE WEB OF *NEPHILA CLAVIPES* (ARANEAE, ARANEIDAE)

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ABSTRACT

The orb web of *Nephila clavipes* has been fractionated into a water soluble and water insoluble fraction. The former contained K^+ (1.8%), Na^+ (0.5%), and ninhydrin reactive amines (20.6%) of the original web weight. The ninhydrin reactive amines were mainly glycine and a gamma-aminobutyric acid related compound.

The water insoluble fraction (fibroin) was digested with trypsin into a soluble and insoluble fraction. Amino acid analysis suggested that the trypsin insoluble fraction derived from the large ampullate gland and thus represents the radial fibers and hub spiral.

These observations are compared to those previously made for *Argiope aurantia* and *Argiope trifasciata.*

INTRODUCTION

Fisher and Brander (1960) first realized that nearly half the weight of the spider's orb web is water soluble. The water soluble fraction includes gamma-aminobutyric acid (GABA) and taurine derivatives as well as K^+, phosphate, and nitrate (Fisher and Brander 1960, Schildknecht et. al. 1972, Andersen and Tillinghast 1980). The phosphate and amines are confined to the sticky spiral of the orb web (Kavanagh and Tillinghast 1979, Andersen and Tillinghast 1980), but their exact role is not known. We have examined the orb web of the subtropical spider, *Nephila clavipes* (Linnaeus) for comparison with the more temperate orb weavers, *Argiope aurantia* Lucas, *Argiope trifasciata* (Forskal), and *Araneus diadematus* Clerck, to observe whether qualitative or quantitative differences might exist and thereby gain insight into their function. Some preliminary observations on the web chemistry of *Latrodectus mactans* (Fabricius) are also presented.

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MATERIAL AND METHODS

Mature female *N. clavipes* were obtained locally (New Orleans, LA) and confined to cages 50 x 50 x 10 cm with glass plates front and back. The spiders were maintained at a constant environment of 18°C and 50% relative humidity on a 12 hour dark/light cycle. The webs constructed were collected daily on glass rods and frozen until analyzed.

Thirty-four webs were collected, pooled, desiccated, and weighed (23.6 mg). The webs were washed twice in 5.0 ml volumes of distilled water to remove water soluble substances. The water insoluble fibroin was removed, desiccated, and weighed (11.5 mg). The web wash was analyzed for pH, water soluble amines by the method of Moore and Stein (1948), inorganic phosphate by the method of Chen et. al. (1956) and protein by the method of Bradford (1976). In addition, samples of web wash were assayed for potassium and sodium using an Instrumentation Laboratory Model 251 flame photometer, as well as amino acids in a Beckman model 118 CL amino acid analyzer. Other samples were first hydrolyzed in 6 N HCl at 110°C for 18 hr prior to automatic amino acid analysis.

The water insoluble fibroin was digested with trypsin (1% of the fibroin weight in 5.0 ml 0.05 M Tris buffer, pH 8.1 containing 10 mM $CaCl_2$; porcine pancreas trypsin, Sigma Chemical Co.) for two hours. The digest was then centrifuged for 10 min at 10,000 x G and the trypsin soluble supernatant decanted. The trypsin insoluble fibroin was then desiccated and weighed (8.7 mg). Samples of both the trypsin soluble and insoluble fractions were hydrolyzed in 6 N HCl for 18 hr at 110°C prior to automatic amino acid analysis.

To observe which fibers were digested by trypsin, a solution (10 mg Porcine pancreas trypsin/ml of 0.05 M tris buffer, pH 8.1 containing 10 mM $CaCl_2$) was applied to sticky spiral/radial and sticky spiral/hub spiral junctions. The results were observed with a Zeiss RA 38 microscope and recorded with Polaroid type 55 P/N, 4 x 5 Land Film.

Mature female *Nephila* were sacrificed, the large ampullate glands removed and the tissue separated from the luminal contents. The latter was hydrolyzed in 6 N HCl prior to automatic amino acid analysis.

L. mactans were obtained from California and maintained in New Hampshire confined individually to large bottles. Web was collected from six unfed spiders over a period of one week and was analyzed for water soluble amines and phosphate.

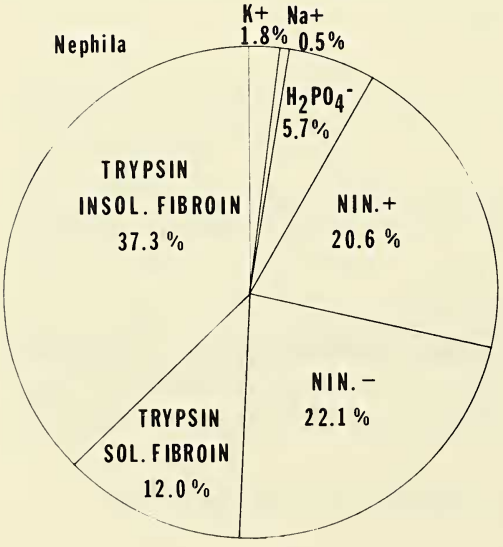

Fig. 1.—The proportional composition by weight of the web of *N. clavipes.*

RESULTS

The 34 webs had a total dry weight of 23.6 mg. Of this 51% was removed by washing in distilled water (water soluble fraction) and 49% remained as water insoluble fibroin.

The water soluble fraction had a pH of 4.9 and contained K^+, Na^+, inorganic phosphate, and ninhydrin reactive amines. The ratio of their occurrence on the orb web is presented in Fig. 1. Automatic amino acid analysis of this fraction revealed two major components, one corresponding to glycine and the second gabamide (Fisher and Brander 1960, Anderson and Tillinghast 1980). Acid hydrolysis prior to automatic amino acid analysis revealed three major components. These corresponded to taurine, glycine, and GABA standards (Fig. 2). These same procedures demonstrated gabamide to be the principle ninhydrin reactive amine on the web of the black widow.

When digested with trypsin, 25% of the water insoluble fibroin was solubilized (*trypsin soluble fibroin*, Fig. 1). The amino acid composition of the trypsin-insoluble fibroin and luminal contents of the large ampullate glands are presented in Table 1. Figure 3 demonstrated that trypsin solubilizes the sticky spiral but not the hub spiral.

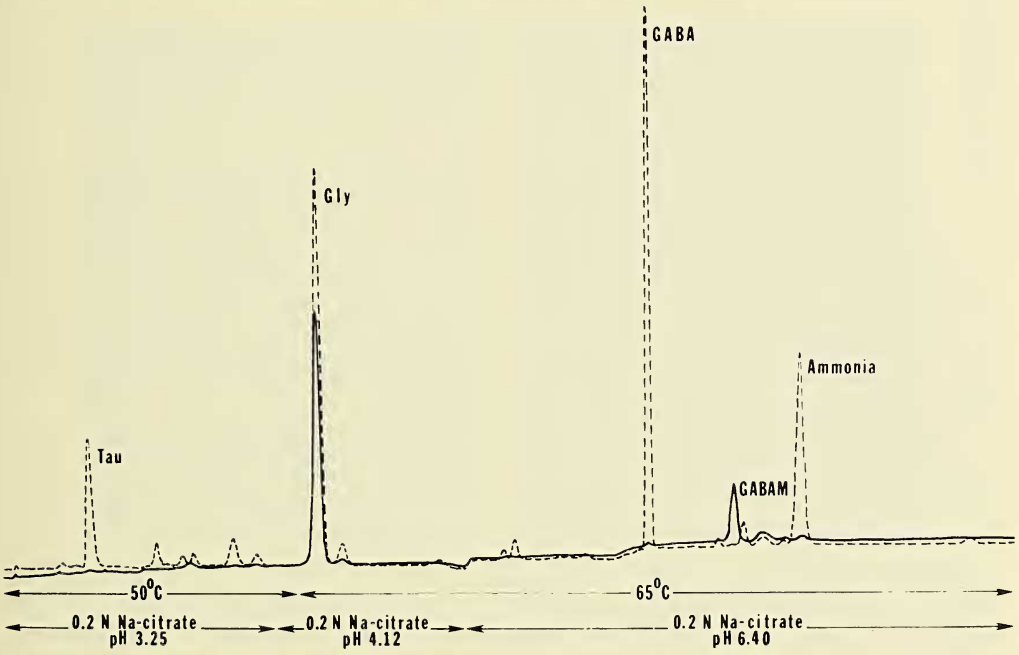

Fig. 2.–A tracing of the amino analysis record of unhydrolyzed (———) and acid-hydrolyzed (– – – –) web washes.

DISCUSSION

The 34 webs gave a total dry weight of 23.6 mg and thus an average weight of 694 ug per web. We have previously recorded web weights for *A aurantia* of 1,533 ug and 386 ug for *A. trifasciata* (Anderson and Tillinghast 1980). It is possible that having confined *Nephila* to cages which are small compared to web size in the field, that slightly atypical webs have been collected and that the ratio of components discussed below may differ somewhat from field constructed web.

Table 1.—The amino acid composition (moles/100 moles) of the contents of the large ampullate glands of *Nephila clavipes* and the trypsin insoluble fraction of its web.

	Large Amp. Gland	Trypsin Insoluble
asp	1.9	1.2
thr	1.0	0.6
ser	3.0	3.8
glu	10.1	10.7
pro	1.7	1.7
gly	40.3	42.7
ala	28.4	26.8
1/2 cys	–	–
val	1.5	1.3
met	0.3	0.1
iso	0.6	0.4
leu	4.5	4.3
nor-leu	–	–
tyr	3.1	3.6
phe	0.5	0.4
his	0.2	0.2
lys	0.8	0.2
ammonia	–	–
trp	–	–
arg	2.0	1.9

Although we recognize that the territories of *N. clavipes* and the two *Argiope* species overlap, we anticipated that the subtropical *Nephila* might exhibit differences in web chemistry from the more northern *Argiope* species. We were surprised, therefore, to observe that actually strong similarities exist. All three species have Na^+, K^+, phosphate, and ninhydrin reactive amines. The ratio of these water soluble substances do differ, but not in a way which is readily related to the environment.

GABA and taurine derivatives are present in the water soluble extract of the web of *N. clavipes* as it is in that of *A. diadematus* (Fisher and Brander 1960) and the *Argiope* species (Anderson and Tillinghast 1980). As we hitherto had thought these compounds to be confined to the webs of orb weavers, we were surprised to observe that the GABA derivative is actually the principal water soluble ninhydrin reactive amine on the web of the black widow. In retrospect, however, our results might have been anticipated for Kovoor and Zylberberg (1979) had hypothesized that K^+, phosphate, and amines are

Table 2.—A comparison of the web fractions of three orb weaving spiders (* = data from Tillinghast, 1983).

	*A. aurantia**	*A. trifasciata**	*Nephila clavipes*
water soluble			
KH_2PO_4	12.6%	5.6%	7.5%
Ninhydrin +	19.8	13.4	20.6
Ninhydrin -	12.6	28.0	22.1
water insoluble			
trypsin sol.	18.0	18.0	12.0
trypsin insol.	37.0	35.0	37.3

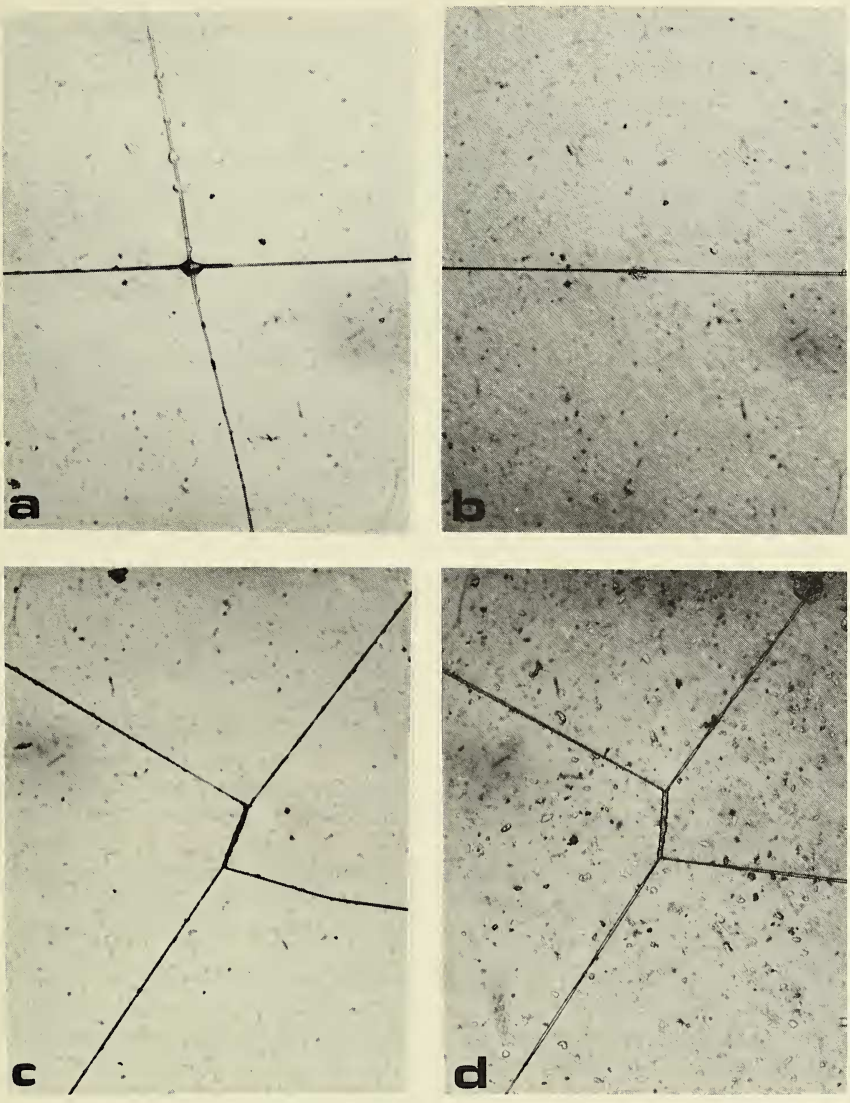

Fig. 3.–The effect of trypsin on the web of *N. clavipes;* (a) before and (b) ten minutes after the addition of trypsin to the sticky spiral/radial junction (Notes the removal of the adhesive spiral fibers); (c) before and (d) two hours after the addition of trypsin to the hub spiral/radial junction.

transferred to the web via the aggregate ducts and these ducts are present in *Nephila* (Peters 1955) as well as *L. mactans* (Kovoor 1977).

Trypsin solubilizes the sticky spiral and stabilimentum of the web of *A. aurantia* (Tillinghast 1983) and it is likely that the trypsin soluble fraction of *Nephila* reported here represents the sticky spiral (Fig. 3). In view of the fact that the hub spiral of *Nephila* is woven between the sticky spiral fibers (see Fig. 341, Kaston 1978) this may account for the higher trypsin insoluble/trypsin soluble ratio (3) found here for *Nephila* than that observed for *Argiope* species (1.9, Tillinghast 1983).

The trypsin insoluble fraction of the orb web probably originates from the large ampullate glands. The amino acid composition of both are nearly identical and agree very reasonably with the data of Zemlin (1968) for drawn silk from this species. Neither radii nor hub spiral are cleaved by trypsin (Fig. 3).

It is of interest to note that the luminal contents of the large ampullate gland of *N. clavipes* has a low content of proline (1.7%, Table 1) compared to that of 10.7% for *A. diadematus* (Andersen 1970) and 11% for *A. aurantia* (Tillinghast 1983). Work (1981) has observed supercontraction ratios for the large ampullate fibers for *A. aurantia* (0.548) and *A. trifasciata* (0.586) to differ from that of *N. clavipes* (0.635). The extent to which the proline content contributes to these differences in supercontraction must await a more detailed analysis.

Finally, whereas a considerable similarity exists as to the composition of water soluble substances on the orb web of all Araneidae thus far studied, the exact ratio of these compounds appears to differ. However, one should view the data presented in Table 2 with caution for these represent composites of a season's collection and give no indication of individual variation of web composition.

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